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(FILE 'HOME' ENTERED AT 07:50:31 ON 16 JAN 2004)

FILE 'DISSABS, IMOBILITY, AGRICOLA, AQUASCI, BIOTECHNO, COMPENDEX,
COMPUAB, CONF, CONFSCI, ELCOM, HEALSAFE, IMSDRUGCONF, LIFESCI, OCEAN,
MEDICONF, PASCAL, PAPERCHEM2, POLLUAB, SOLIDSTATE, ADISCTI, ADISINSIGHT,
ADISNEWS, ANABSTR, BIOBUSINESS, BIOCOMMERCE, ...' ENTERED AT 07:50:43 ON
16 JAN 2004

L1 2760 S (SCAPULOPERONEAL MUSCULAR DYSTROPHY) OR SPMD OR (HYALINE BODY
L2 10 S L1 (S) ((ALPHA (A) 7) OR (ALPHA7) OR (ALPHA (A) 7A) OR (BETA
L3 7 DUP REM L2 (3 DUPLICATES REMOVED)
E KAUFMAN-STEPHEN.IN.
E KAUFMAN-STEPHEN?/AU
E KAUFMAN STEPHEN?/AU
L4 23 S E1 OR E2
L5 0 S L1 AND L4

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L3 ANSWER 1 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN

ACCESSION NUMBER: 2003-03030 BIOTECHDS

TITLE: Identifying individual exhibiting symptoms of muscular dystrophy, for diagnosing and treating muscular dystrophy, by detecting transcription or translation product of alpha7beta1 integrin gene in a tissue sample;
virus vector or plasmid-mediated gene transfer and expression in stem cell or myoblast for Duchenne muscular dystrophy diagnosis and gene herapy

AUTHOR: KAUFMAN S J

PATENT ASSIGNEE: UNIV ILLINOIS FOUND

PATENT INFO: WO 2002066989 29 Aug 2002

APPLICATION INFO: WO 2002-US6376 20 Feb 2002

PRIORITY INFO: US 2001-286890 27 Apr 2001; US 2001-270645 20 Feb 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2002-674967 [72]

AB DERWENT ABSTRACT:

NOVELTY - Identifying (M1) symptoms of muscular dystrophy (MD) in individual suffering from **scapuloperoneal muscular dystrophy (SPMD)**, comprises detecting a transcription or translation product of an alpha7beta1 integrin gene in a tissue sample.

DETAILED DESCRIPTION - Identifying (M1) symptoms of muscular dystrophy (MD) in individual suffering from **scapuloperoneal muscular dystrophy (SPMD)**, comprises detecting a transcription or translation product of an alpha7beta1 integrin gene in a tissue sample. (M1) comprises: (a) obtaining a tissue sample from an individual exhibiting symptoms of a dystrophy, where the sample is obtained from a tissue known in a normal individual to express alpha7beta1 integrin; (b) detecting a transcription or translation product of an alpha7beta1 integrin gene in the sample; and (c) determining a level of the transcription or translation product of the alpha7beta1 integrin gene in the sample as compared with a level in a tissue sample from the same tissue of a normal individual. **SPMD** is diagnosed when the tissue sample of the individual exhibiting MD symptoms, comprises a level of a transcription or translation product of the alpha7beta1 integrin gene in the tissue sample that is lower than the level in a tissue sample from the same tissue of a normal individual.

INDEPENDENT CLAIMS are also included for: (1) a reporter gene construct (I) comprising a transcription regulatory sequence of a human **alpha7** integrin gene and a reporter coding sequence; (2) a recombinant host cell (II) comprising the reporter gene construct; (3) identifying (M2) a composition that increases expression of an **alpha7** integrin gene, comprises: (a) contacting the recombinant host cell with a test composition to produce a contacted recombinant host cell; (b) monitoring reporter coding expression in the contacted recombinant host cell and monitoring expression of the reporter coding sequence of the reporter gene construct in a recombinant host cell that has not been contacted with the test composition; and (c) determining if the test composition increases reporter coding sequence expression when the expression of the reporter coding sequence is greater in the contacted host cell than in the recombinant host cell that has not been contacted with the test composition, where a composition that increases the expression of an **alpha7** integrin gene is identified when the expression of the reporter coding sequence is greater in the contacted host cell than in the recombinant host cell that has not been contacted with the test composition; (4) alleviating (M4) symptoms of MD having: (a) **alpha7** integrin levels that are lower in a patient suffering from or susceptible to MD than in a normal individual, comprises administering to the patient the composition identified by (M3);

or (b) levels of **alpha7** integrin, dystrophin and/or utrophin that are lower in a patient suffering from or susceptible to MD than in a normal individual, comprises administering to the patient a DNA construct comprising an **alpha7** integrin coding sequence operably linked to a transcription regulatory sequence that enables selective expression in muscle cells and a vector sequence.

BIOTECHNOLOGY - Preferred Method: The translation product of an **alpha7beta1** integrin gene in the tissue sample is detected by contacting the tissue sample using an **alpha7beta1** integrin-specific antibody that is detectably labeled. A transcription product of an **alpha7beta1** integrin gene is detected in the tissue sample using reverse transcriptase-polymerase chain reaction (RT-PCR). The primers used in the RT-PCR comprise a sequence of (S4) and (S5). In (M2), where the monitoring and determining steps are carried out in high throughput assay format. In the method of (4), where the MD is Duchenne muscular dystrophy. The vector sequence is a virus vector sequence or a plasmid sequence. Administering comprises ex vivo transformation of stem cells or myoblasts isolated from the patient to produce transformed myoblasts and subsequent administration of the transformed stem cell or transformed myoblasts to the patient with the result that the transformed myoblasts differentiate to form muscle cells that express **alpha7** integrin, where the symptoms of MD is ameliorated. **Preferred Gene Construct:** The reporter coding sequence is selected from the group of a green fluorescent protein, luciferase, beta-lactamase, beta-galactosidase, or beta-glucuronidase, or an immunological tag portion. The transcription regulatory sequence comprises a sequence of 1970 base pairs fully defined in the specification. The reporter gene construct further comprises a vector sequence. **Preferred Host Cell:** The cell is preferably a cultured muscle cell. GAACAGCACCTTTCTGGAGG (S4) CCTTGAAGTCTGTCGGTCT (S5)

ACTIVITY - Inotropic. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - (M1-4) are useful for diagnosing, ameliorating and treating muscular dystrophy symptoms such as **scapuloperoneal muscular dystrophy** or Duchenne muscular dystrophy. The nucleic acid probes, primers or immunological probes can be used for detecting the reduction of or lack of expression of the **alpha7beta1** integrin in **SPMD**.

ADMINISTRATION - Administration may be intravenous, intramuscular or by regional perfusion (all claimed). No dosage details given.

EXAMPLE - No suitable example given. (53 pages)

L3 ANSWER 2 OF 7 IFIPAT COPYRIGHT 2004 IFI on STN DUPLICATE 2
AN 10249003 IFIPAT;IFIUDB;IFICDB
TITLE: DIAGNOSTICS, ASSAY METHODS AND AMELIORATION OF
MUSCULAR DYSTROPHY SYMPTOMS
INVENTOR(S): Kaufman; Stephen J., Urbana, IL, US
PATENT ASSIGNEE(S): Unassigned
AGENT: GREENLEE WINNER AND SULLIVAN P-C, 5370 MANHATTAN
CIRCLE, SUITE 201, BOULDER, CO, 80303 US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2002192710	A1	20021219
APPLICATION INFORMATION:	US 2002-81885		20020220

	NUMBER	DATE
PRIORITY APPLN. INFO.:	US 2001-270645P	20010220 (Provisional)
	US 2001-286890P	20010427 (Provisional)
FAMILY INFORMATION:	US 2002192710	20021219
DOCUMENT TYPE:	Utility	
	Patent Application - First Publication	

** 00001000

FILE SEGMENT: CHEMICAL

APPLICATION

GOVERNMENT INTEREST:

(0002) This invention was made, at least in part, with funding from the National Institutes of Health. Accordingly, the United States government has certain rights in this invention.

NUMBER OF CLAIMS: 23 12 Figure(s).

DESCRIPTION OF FIGURES:

FIGS. 1A-1D illustrate the genotyping of transgenic alpha 7BX2mdx/utr (-/-) mice. FIG. 1A: The alpha 7BX2 transgene (tg) was detected by PCR using primers that amplify between the MCK promoter and the **alpha 7** cDNA sequence. Lanes 2 and 3 are positive for the MCK-alpha 7BX2 transgene. FIG. 1B: Southern analysis using a rat **alpha 7** specific probe of EcoRI and KpnI digested genomic DNA. The 7.1 kb band corresponding to the rat transgene construct is detected in lanes 4, 5 and 6. A higher 14.2 kb transgene dimer was also detected. Samples in these lanes are from mdx/utr (-/-) mice. DNA in lanes 1, 2 and 3 are from non-transgenic mice. FIG. 1C: Determining the status of the utrophin gene by PCR. Only mutant utr alleles are detected in lanes 1 and 4 identifying utr (-/-) mice. One wildtype (wt) and one mutant allele are amplified in lane 2, identifying a utr (+/-) mouse. Lane 3 is wildtype at both utr loci. FIG. 1D: Determining the status of the dystrophin gene by PCR. The mdx primer set detects the point mutation in the dystrophin gene, whereas the wt primers detect only the wildtype allele. Mouse 2 is wildtype at the dystrophin locus, mouse 3 is heterozygous (mcW+) and mouse 4 is mdx. Lane 1 contains no DNA.

FIG. 2 demonstrates the expression of the rat **alpha 7** protein in mouse muscle. Immunofluorescence analysis of hindlimb cryosections using monoclonal antibodies against the rat **alpha 7** integrin chain, dystrophin, and utrophin. AChRs were stained with rhodamine-labeled alpha-bungarotoxin. The rat **alpha 7** protein is only detected in transgenic mice and localizes to the membrane of muscle fibers. The lack of dystrophin and utrophin in both transgenic and non-transgenic mdx/utr (-/-) mice confirms their genotypes.

FIG. 3 illustrates the immunofluorescence of **beta 1** integrin isoforms in the hindlimb of 8 week wildtype, mdx, mdx/utr (-/-) and alpha 7BX2-mdx/utr (-/-) mice. beta 1A integrin is elevated in muscle fibers of mdx/utr (-/-) mice compared to wildtype and mdx animals. In contrast, beta 1A levels are normal in alpha 7BX2-mdx/utr (-/-) mice. Compared to wildtype, an increase in beta 1D is detected in both mdx and mdx/utr (-/-) muscle. alpha 7BX2-mdx/utr (-/-) mice show an additional increase in beta 1D compared to both mdx and mdx/utr (-/-) mice.

FIGS. 4A-4C show the transgenic expression of alpha 7BX2 increases the amount of beta 1D in hindlimb muscle. FIG. 4A: Western blot showing more alpha 7B is detected in transgenic mice compared to non-transgenic mice whereas ***alpha*** 7A is constant. FIG. 4B: The blots were re-probed with anti creatine kinase antibody. The CK levels were used to normalize the amounts of **alpha 7A** and alpha 7B proteins in each sample. The levels of **alpha 7A**/CK in both transgenic and nontransgenic mice remained constant. In contrast, alpha 7B/CK ratio is 2.3 fold higher in the alpha 7BX2 transgenic mice compared to the nontransgenic animal. FIG. 4C: beta 1D integrin from 8 week hindlimb muscle. Less beta 1D is detected in mdx/utr (-/-) mice compared to alpha 7BX2-mdx/utr (-/-) mice. An increase of approximately 1.5-fold more beta 1D was detected in the transgenic vs non-transgenic mice.

FIG. 5 provides Kaplan-Meier survival curves of 43 alpha 7BX2mdx/utr (-/-) and 84 mdx/utr (-/-) mice. Wilcoxon and Log rank tests show the alpha 7BX2-mdx/utr (-/-) mice and mdx/utr (-/-) populations have distinct survival curves (P less-than 0.001). The alpha 7BX2-mdx/utr (-/-) mice survive 3-fold longer than non-transgenic mdx/utr (-/-) mice with a median life expectancy of 38 weeks. In contrast, non-transgenic mdx/utr (-/-) mice have a median life expectancy of just 12 weeks. 95% confidence intervals are indicated by shading.

FIG. 6 illustrates weight gain vs survival in representative mdx/ utr (-/-) mice and alpha 7BX2-mdx/utr (-/-) mice. The majority of non-transgenic mdx/utr (-/-) mice undergo a crisis at 5-10 weeks of age that results in a sudden loss

of weight and premature death. Most transgenic mdx/utr (-/-) mice live longer and maintain weight. Eventually these also go through a crisis that results in weight loss.

FIG. 7 shows histology of hindlimbs from 10 week wildtype, mdx, mdx/utr (-/-) and alpha BX2-mdx/utr (-/-) mice. Hematoxylin and eosin staining reveal abundant central nuclei in mdx, mdx/utr (-/-) and alpha 7BX2-mdx/utr (-/-) mice. Mononuclear cell infiltration and expression of fMyHC are extensive in the mdx/utr (-/-) mice, but are reduced in the alpha 7BX2-mdx/utr (-/-) transgenic animals, indicating less degeneration and more stable regeneration in these mice.

FIG. 8 shows the results of X-ray and magnetic resonance imaging of normal and dystrophin mice. Upper panels: the severe spinal curvature (kyphosis) and constriction of the rib cage in mdx/utr (-/-) mice are largely reduced in the alpha 7BX2 transgenic animals. Lower panels: MRI of mid-sagittal sections reveal kyphosis and reduction of pulmonary volume in mdx/utr (-/-) mice are largely alleviated in transgenic mice.

FIG. 9 show that severe spinal curvature (kyphosis) and hindlimb claspings (joint contractures) are largely reduced in mice expressing the rat alpha 7BX2 transgene.

FIG. 10 provides en face images of neuromuscular junctions of 8 week wildtype, mdx/utr (-/-) and alpha 7BX2-mdx/utr (-/-) mice. Localization of acetylcholine receptors (AChRs) in the postsynaptic membrane of wildtype mice, detected with rhodaminelabeled alpha-bungarotoxin, is continuous and uninterrupted. In contrast, mdx/utr (-/-) mice have discontinuous distributions of AChRs organized into discrete "boutons". The organization of the postsynaptic membrane in alpha 7BX2-mdx/utr (-/-) transgenic mice has a more continuous (normal) en face pattern.

FIG. 11 documents PCR detection of integrin **alpha 7A** and **alpha 7B** in normal control and **SPMD** patient samples. 35 cycles of amplification reveal minimal amounts of **alpha 7A** in the

SPMD patient samples.

FIG. 12 presents an immunofluorescence analysis of muscle biopsy material from three **SPMD** patients. The **alpha 7A** integrin is absent from all three patient samples, and the amount of **alpha 7B** is decreased in relation to the severity of the pathology in the patients. Interestingly, the amounts of dystrophin and beta -dystroglycan, two proteins that comprise an alternative adhesive mechanism, is increased in the **SPMD** patient biopsy materials.

AB The present disclosure provides compositions and sequences for the diagnosis, genetic therapy of certain muscular dystrophies, especially muscular dystrophy resulting from a deficiency in dystrophin protein or a combined deficiency in dystrophin and utrophin, and methods and compositions for the identification of compounds which increase expression of the **alpha 7** integrin. Expression of the integrin alpha BX2 polypeptide in muscle cells results in better physical condition in a patient or an animal lacking normal levels of dystrophin or dystrophin and utrophin. The present disclosure further provides immunological and nucleic acid based methods for the diagnosis of **scapuloperoneal muscular dystrophy**, where there is a reduction in or absence of **alpha 7A** integrin expression in muscle tissue samples and normal levels of laminin-(fraction (2/4)) in those same samples. The present disclosure further provides methods for identifying compositions which increase the expression of **alpha 7** integrin protein in muscle cells of dystrophy patients.

L3 ANSWER 3 OF 7 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: ABQ80576 DNA DGENE

TITLE: Identifying individual exhibiting symptoms of muscular dystrophy, for diagnosing and treating muscular dystrophy, by detecting transcription or translation product of alpha7beta1 integrin gene in a tissue sample -

INVENTOR: Kaufman S J

PATENT ASSIGNEE: (UNII)UNIV ILLINOIS FOUND.

PATENT INFO: WO 2002066989 A2 20020829 53p
APPLICATION INFO: WO 2002-US6376 20020220
PRIORITY INFO: US 2001-270645P 20010220
US 2001-286890P 20010427
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 2002-674967 [72]
DESCRIPTION: Human alpha7 integrin promoter region.
AB The present invention relates to a method for identifying symptoms of muscular dystrophy (MD) in individual suffering from **scapuloperoneal muscular dystrophy (SPMD)**. The method comprises detecting a transcription or translation product of an alpha7beta1 integrin gene in a tissue sample. The present sequence is the human **alpha7** integrin promoter region. This sequence also comprises part of exon 1. The human **alpha7** integrin transcription regulatory sequences were identified as part of the Homo sapiens chromosome 12 BAC, RP11-644F5. This sequence was used to generate reporter constructs in an example from the invention.

L3 ANSWER 4 OF 7 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: ABQ80575 DNA DGENE
TITLE: Identifying individual exhibiting symptoms of muscular dystrophy, for diagnosing and treating muscular dystrophy, by detecting transcription or translation product of alpha7beta1 integrin gene in a tissue sample -
INVENTOR: Kaufman S J
PATENT ASSIGNEE: (UNII)UNIV ILLINOIS FOUND.
PATENT INFO: WO 2002066989 A2 20020829 53p
APPLICATION INFO: WO 2002-US6376 20020220
PRIORITY INFO: US 2001-270645P 20010220
US 2001-286890P 20010427
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 2002-674967 [72]
DESCRIPTION: Human alpha7 integrin PCR primer hu3438R.
AB The present invention relates to a method for identifying symptoms of muscular dystrophy (MD) in individual suffering from **scapuloperoneal muscular dystrophy (SPMD)**. The method comprises detecting a transcription or translation product of an alpha7beta1 integrin gene in a tissue sample. A panel of overlapping primers were designed from the **alpha7** cDNA sequence, and were used in RT-PCR to screen patient RNA for transcriptional expression of the integrin alpha7A subunit isoform. Primers ABQ80574 and ABQ80575 were used to amplify around the human a7A/a7B alternative splice site. It was found that in **SPMD** patients there is very little alpha7A amplification product in comparison to the amount seen in a normal individual.

L3 ANSWER 5 OF 7 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: ABQ80574 DNA DGENE
TITLE: Identifying individual exhibiting symptoms of muscular dystrophy, for diagnosing and treating muscular dystrophy, by detecting transcription or translation product of alpha7beta1 integrin gene in a tissue sample -
INVENTOR: Kaufman S J
PATENT ASSIGNEE: (UNII)UNIV ILLINOIS FOUND.
PATENT INFO: WO 2002066989 A2 20020829 53p
APPLICATION INFO: WO 2002-US6376 20020220
PRIORITY INFO: US 2001-270645P 20010220
US 2001-286890P 20010427
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 2002-674967 [72]

DESCRIPTION: Human alpha7 integrin PCR primer hu3101F.

AB The present invention relates to a method for identifying symptoms of muscular dystrophy (MD) in individual suffering from **scapuloperoneal muscular dystrophy (SPMD)**. The method comprises detecting a transcription or translation product of an alpha7beta1 integrin gene in a tissue sample. A panel of overlapping primers were designed from the **alpha7** cDNA sequence, and were used in RT-PCR to screen patient RNA for transcriptional expression of the integrin alpha7A subunit isoform. Primers ABQ80574 and ABQ80575 were used to amplify around the human a7A/a7B alternative splice site. It was found that in **SPMD** patients there is very little alpha7A amplification product in comparison to the amount seen in a normal individual.

L3 ANSWER 6 OF 7 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: ABQ80572 DNA DGENE

TITLE: Identifying individual exhibiting symptoms of muscular dystrophy, for diagnosing and treating muscular dystrophy, by detecting transcription or translation product of alpha7beta1 integrin gene in a tissue sample -

INVENTOR: Kaufman S J

PATENT ASSIGNEE: (UNII)UNIV ILLINOIS FOUND.

PATENT INFO: WO 2002066989 A2 20020829 53p

APPLICATION INFO: WO 2002-US6376 20020220

PRIORITY INFO: US 2001-270645P 20010220

US 2001-286890P 20010427

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: 2002-674967 [72]

DESCRIPTION: Rat alpha7BX2 integrin PCR primer AATII.

AB The present invention relates to a method for identifying symptoms of muscular dystrophy (MD) in individual suffering from **scapuloperoneal muscular dystrophy (SPMD)**. The method comprises detecting a transcription or translation product of an alpha7beta1 integrin gene in a tissue sample. To illustrate the method, a mouse Muscle Creatine Kinase (MCK) promoter-rat alpha7BX2 integrin construct was made. The construct was then used to produce transgenic mdx/utr(-/-) mice. Primers ABQ80571 and ABQ80572 used to amplify between the MCK promoter and the **alpha7** integrin cDNA resulted in a 455bp amplicon only in transgenic mice.

L3 ANSWER 7 OF 7 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: ABQ80571 DNA DGENE

TITLE: Identifying individual exhibiting symptoms of muscular dystrophy, for diagnosing and treating muscular dystrophy, by detecting transcription or translation product of alpha7beta1 integrin gene in a tissue sample -

INVENTOR: Kaufman S J

PATENT ASSIGNEE: (UNII)UNIV ILLINOIS FOUND.

PATENT INFO: WO 2002066989 A2 20020829 53p

APPLICATION INFO: WO 2002-US6376 20020220

PRIORITY INFO: US 2001-270645P 20010220

US 2001-286890P 20010427

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: 2002-674967 [72]

DESCRIPTION: Mouse muscle creatine kinase promoter PCR primer MCK1.

AB The present invention relates to a method for identifying symptoms of muscular dystrophy (MD) in individual suffering from **scapuloperoneal muscular dystrophy (SPMD)**. The method comprises detecting a transcription or translation product of an alpha7beta1 integrin gene in a tissue sample. To illustrate the method, a mouse Muscle Creatine Kinase (MCK) promoter-rat alpha7BX2 integrin construct was made. The construct was

then used to produce transgenic mdx/utr(-/-) mice. Primers ABQ80571 and ABQ80572 used to amplify between the MCK promoter and the **alpha7** integrin cDNA resulted in a 455bp amplicon only in transgenic mice.